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Role of Eosinophils Apoptosis and Phagocytosis in Sinonasal Polyps: An Immunohistochemical and Ultrastructural Study

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Abstract

Background: Inflammatory cells disappear from airway tissue through apoptosis and phagocytosis . However , the cells may also be cleared through primary cytolysis , necrosis secondary to apoptosis or transepithelial migration .

Material and Methods: This study evaluated the role of apoptosis and phagocytosis of tissue eosinophils in the sinonasal polyps received from fifty patients . Immunohistochemically , the amount of CD 95 — and CD 68 – positive cells was determind by counting the average number in ten randomly chosen high power fields . The inflammatory infiltrate consisted of numerous eosinophils as well as a considerable amount of lymphocytes and macrophages . Also, we detected a frequent expression of CD 95 on the eosinophils and numerous other inflammatory cells .

Results: CD 68 – positive cells (most probably macrophages) approximately equalled the number of eosinophils . Ultrastructurally , seventy three percent of the examined eosinophils (365 / 500) were apoptotic ; about one third of which (89 / 365 , 24.3 %) were secondarily necrosed . However , only four percent (20 / 500) were cytolytic . The phagocytic action of macrophages engulfing eosinophils was confirmed by electron microscopy .

Conclusion: Thus , our study concluded the elimination of tissue eosinophils predominently through apoptosis and phagocytosis that might be important for clearance of eosinophils from the respiratory tissue and could improve the allergic manifestations .

Key Wards : Sinonasal polyps – Apoptosis – Phagocytosis – Eosinophils – Macrophages – CD 95 (Fas receptor) – CD68 – Immunohistochemistry – Electron Microscopy .

Introduction

Sinonasal polyps are non-neoplastic swellings usually arising from the ethmoid sinuses and prolapse into the nasal cavity . Previous studies had been concerned the etiology , the relation to allergy , histopathology and treatment of these lesions (Hellquist, 1996), however, a little attention about apoptosis and resolution of the eosinophilic inflammatory infiltrate in these polyps had been performed (Davidsson *et al* ., 2000) .

The polyps characteristically contain a leucocyte infiltrate in which eosinophils are often remarkably prominent (Jahnsen *et al* ., 1995) The later cells are bone marrow derived granulocytes with a richness of surface receptors for immunologic ligands (Rothenberg , 1998) . They harbor specific granules which, beside chemokines, cytokines, and growth factors , contain large amounts of tissue – toxic cationic proteins namely major basic protein , eosinophil – derived neurotoxin , eosinophil cationic protein (ECP) and eosinophil peroxidase (Martin *et al.*, 1996). Moreover, eosinophils produce tumor necrosis factor – alpha (TNF – ∞) , transforming growth factor – alpha (TGF – ∞) and transforming growth factor – beta 1 (TGF – β1) . Thus , these cells actively contribute to mucosal inflammation and may cause several morphological changes occurring in sinonasal polyps . The demonstration of m RNA for interleukin (IL) -6 & IL – 12 can

imply T-helper 1 cell response (Davidsson *et al*.,1996). Endothelial adhesion molecules particularly vascular cell adhesion molecule – 1 (VCAM -1) and P-selectin are thought to play a crucial role in human eosinophil extravasation at inflammatory sites and nasal polyps (Karlsson and Hellquist, 1996, Desreumaux and Capron , 1996 & Westergren *et al* ., 1997). In addition, eosinophils abounding and degranulation in airway mucosal and pulmonary tissues are belived to have pathogenic roles in asthma, nasal polyposis, allergic rhinitis and eosinophilic pneumonia (Erjefalt and Persson , 2000 , Erjefalt *et al* ., 2001 & Emanuelsson *et al* ., 2004). During active seasonal allergic rhinitis eosinophils may exhibit a more pronounced degranulation than in many other common diseases characterized by tissue eosinophilia e.g. inflammatory bowel disease and asthma (Erjefalt *et al* ., 1998 & Erjefalt *et al* ., 2001) . However , a little information is known about the dynamics of eosinophilic degranulation in allergic rhinitis especially when the disease progresses from an asymptomatic baseline situation into an active symptomatic condition (Emanuelsson *et al* ., 2004).

Infiltration of leucocytes in diseased airway tissues is counterbalanced by their elimination through apoptosis (Woolley *et al*., 1996) and prompt engulfment by macrophages (Haslett , 1999 & Henson *et al* ., 2001) . In vitro study has generated a detailed information regarding molecular and pharmacologic regulation of apoptosis (Nutku *et al*., 2003) . Human purified blood eosinophils seem to be prone to massive apoptosis particularly when cultured in the absence of specific growth factors or in the presence of glucocorticoids in the cell medium (Gardai *et al* ., 2003 & Bochner , 2004) . Steroid administration in vitro has been shown to significantly inhibit the survival rate of eosinophils from patients with atopic dermatitis and has suggested that steroid – induced apoptosis decreases the eosinophil count, in vivo , in these patients (Matsukura *et al* ., 1997).

Further support for the role of apoptosis is provided by demonstration of Fas-receptor (CD 95 ; APO -1) on lung eosinophils (Tsuyuki *et al* ., 1995) and by the delay of neutrophils apoptosis mediated by interleukin-6 (Biffi *et al* , 1996).

Moreover, the production in nasal polyps of ILs-3, -5& -8 as well as granulocyte –macrophage colony – stimulating factor (GM – CSF) & TNF - ∞ indicates a possible involvement of these cytokines in the promotion of eosinophil survival probably by preventing or delaying apoptosis (Bachert *et al* ., 1997) . While the biological activities of IL-3 and GM – CSF extend to affect several cell types , IL-5 acts specifically on eosinophils to promote their maturation , activation and survival (Pretolani and Goldman , 1997 & Till *et al* ., 1997) .

Monocytes and macrophages are certainly different regarding the induction of apoptosis ; monocytes spontaneously undergo apoptosis after cytokine withdrowal whileas macrophages have no great potential to undergo apoptosis (Davidsson *et al* ., 2000) . The later cells from different sources and at various stages of activation may change in their ability to engulf apoptotic cells suggesting the possibility that the dead cells would not be immediately eradicated from all tissues (Uller *et al*., 2004). Furthermore , the occurrence of apoptotic eosinophils and neutrophils has been reported in human skin indicating that the apoptotic cells in vivo should be well detectible by careful histologic examination of tissue (Ying *et al* ., 1997 & Erjefalt and Persson , 2000)

A clear identification of morphologic characteristics , achievable exclusively by transmission electron microscopy (TEM) analysis , is considered crucial for the assessment of occurrence of apoptosis of cells in vivo by specifically appearance of eosinophil cell debris within the macrophages as would be the case if apoptotic eosinophils had been engulfed in vivo (Stadelmann *et al* ., 2000 & Uller *et al* ., 2004) . In addition , TEM analysis further involves the detection of other features of the tissue eosinophils such as occurrence of cytolysis as well as necrosis secondary to apoptosis (Erjefalt *et al* ., 1999 & Uller *et al* ., 2004) .

We , thus , aimed to investigate the role of apoptosis and phagocytosis of tissue eosinophils in the sinonasal polyps .

Material and methods

The material of this work is consisted of fifty sinonasal polyps obtained by routine polypectomy from Otorhinolaryngology Department, Al-Hussein University Hospital during the period from August 2006 to August 2008 . All patients were males with a ges ranged from 28 to 51 years (mean , 42.8±1.7) . All patients had received a local steroid treatment .

Small pieces of the specimens were used for ultrastructural study while the remaining tissue specimens were immediately placed in 4 % neutral buffered formaldehyde and cut up within the next 24 hours . The following techniques were carried out :

1) Routine histopathologic technique :
Formalin fixed and paraffin embedded blocks were sectioned at 6 μm thickness and examined microscopically using hematoxylin and eosin (Hx .& E) stain to ensure the integrity of specimens before the immunoperoxidase technique and to confirm the clinical diagnosis .

2) Immunoperoxidase (IP) technique :
According to Hellquist *et al* ., (1997) & Sundelin et al ., (1997) the sections were deparafinized, hydrated and incubated with 3% H_2O_2 for 5 minutes to block the endogenous peroxidase activity and then immersed in boiling citrate buffer (PH6) in a microwave oven , twice , for 5 minutes (600 W) . After rinsing in Tris buffered saline (TBS) , the sections were incubated with monoclonal primary antibodies for CD 95 (Anti – Fas , Clone UB-2 , Oncor , Gaithersburg , MD) at a dilution of 1:25 and for CD68 (Anti–CD68 , Clone PG – M1 , Dako , Copenhagen , Denmark) at a dilution of 1:150 for 1 hour at room temperature . Then after rinsing in distilled water & TBS buffer , secondary antimouse antibody using peroxidase labelled biotion streptavidin complex detection system (Strept AB complex / AP , Dako , Copenhagen , Denmark) for CD 95 and CD 68 was applied . Diaminobenzidine was added as a chromogen . Then , the sections were counterstained with hematoxylin and coversliped .

Immunohistochemical analysis :

CD 68 – positive cells (most probably macrophages) and CD 95 – positive eosinophils count were evaluated by counting their average numbers in ten randomly selected high power fields (HPF ; X 400 magnification) per polyp . The average number of either positive cells was scored as follows ; O : negative , 1+ : < 5 positive cells , 2 + : 5 - < 20 , and 3+ : ≥ 20 cells per field . CD68 immunopositivity was detected as a brownish nuclear staining while CD95 immunoreactivity appeared as a brownish membranous and cytoplasmic signalling .

3) Transmission electron microscopy (TEM) :
According to Uller et al . (2001) and Erjefalt et al. (2003) , samples for TEM study were prepared . Small pieces of biopsies were put in 2.5 % gluteraldehyde , then in cacodylate buffer for 20 minutes and fixed in a fume cupboard with osmium tetroxide for 2 hours at 4°C . The specimens were dehydrated , cleared in propylene oxide for 30 minutes at room temperature and embedded in Beem capsule filld completely with epoxyresin and polymerized at 60 °C for 24 hours . The capsule was then trimmed and sectioned at 1 μm (Semithin section) and go nm (Ultrathin sections) . The sections were placed on a 200 – mesh , thin – bar , copper grid and contrasted by staining in uranyl acetate (4% in distilled water for 30 minutes at 4°C) and with lead citrate (0.5 % 1M sodium hydroxide for 3 mintes at room temperature). The ultrastructural analysis was carried out using a Philips CM10 TEM (Philips , Eindhoven , Netherlands) .

Ultrastructural analysis :

The biopsies that contained sufficient numbers of eosinophils and had a well – preserved morphology without mechanical artifacts were thirty five out of the fifty cases (35 / 50 , 70 %) . In these appropriate biopsies , each individual eosinophil was carefully analysed at x

4,000 – x 12,000 magnification . In total , we examind 500 eosinophils .

Results .

All biopsies displayed a typical morphology in the routinely stained sections including oedema , excessive inflammatory cell infiltrate , basement membrane thickening and goblet cell proliferation (Fig .1) . The inflammatory infiltrate consisted of numerous eosinophils as well as considerable number of lymphocytes and macrophages that are almost entirely found towards the periphery of the polyps (Fig.2)

Fas receptor (CD 95) - positive eosinophils were found in most cases (46 out of 50 ; 92 %) . Regarding the average number of CD 95 + ve eosinophils , 29 cases (29 / 50 ; 58 %) were of grade 3 +(Fig .3) ,8 (16 %) were of grade 2 + and 9 (18 %) were of grade 1 + (Fig 4) . The remaining cases (4 / 50 ; 8 %) didn't express CD95 on eosinophils (Table 1) . In addition , CD95 molecule was expressed on numerous other inflammatory cells which had not been counted in this study .

CD 68 - positive cells approximately equalled the number of eosinophils . They are detected in all cases although their incidence differed from one polyp to another . Their average number was graded as 3 + in 28 (56 % ; Fig . 5) , 2 + in 12 (24%) and 1 + in 10 (20 % ; Fig . 6) cases (Table 2) . In most cases (46 out of 50 ; 92 %) , CD 95 - positive eosinophils & CD 95 + ve other inflammatory cells appeared more frequent towards the periphery compared to the central part of the polyp (Fig . 7) . The immunohistochemical results , regarding the susceptible CD 95 positive eosinophils for programmed cell death , were confirmed by their apoptotic ultrastructural morphology (Fig . 8A-D) . Eosinophils ultrastructurally were distributed in the epithelium and subepithelial layer of the polyp . Four percent of TEM – examined tissue eosinophils (20 / 500) were cytolytic as detected by incomplete chromatolysis , cell membrane disruption and partly dissolved cytoplasm with spilling of protein – containing free granules . On the other hand , seventy three percent (365 / 500) of the tissue eosinophils were clearly apoptotic with appearance of electron – dense granules , condensed chromatin , nuclear bleb formation , preserved plasma membrane and non – dilated organelles . Secondary necrosis , as a final stage of apoptosis , was encountered in 24 . 3 % of the apoptotic eosinophils (89 / 365) and was distinguished from cytolysis as shown by a condensed dark nucleus as well as plasma and organellar membranes rupture . Thus , most of tissue eosinophils were died by apoptosis rather than by cytolysis (Table 3) In addition , macrophages exhibited signs of engulfment of cell material definitely eosinophils as shown by their specific granules .

Table 1 : Immunoreactivity of eosinophils for CD 95 in studied polyps (n = 50):

CD95 - positive eosinophils			CD95 - negative eosinophils		
No , of cases	%	grade	No , of cases	%	grade
29	58	3 +	4	8	0
8	16	2 +			
9	18	1 +			
Total= 46	92		4	8	

Table 2 : Immunoreactivity of macrophages for CD 68 in studied polyps (n = 50) :

CD95 - positive cells			CD95 - negative cells		
No , of cases	%	grade	No , of cases	%	grade
28	56	3 +	0	0	0
12	24	2 +			
10	20	1 +			
Total= 50	100		0	0	

Table 3 : Ultrastructural findings in TEM – examined eosinophils (n = 50):

Finding	No ; of cells	%
Cytolysis	20	4
Apoptosis only	276	48.7
Apoptosis with secondarynecrosis	89	24.3
	365	73

Fig 1 : A case of sinonasal polyp showing oedema , excessive inflammatory cell infiltrate & basement membrane thickening . Fig 2 : A sinonasal polyp showing an inflammatory infiltrate almost found towards the periphery (H x & E x 200) .

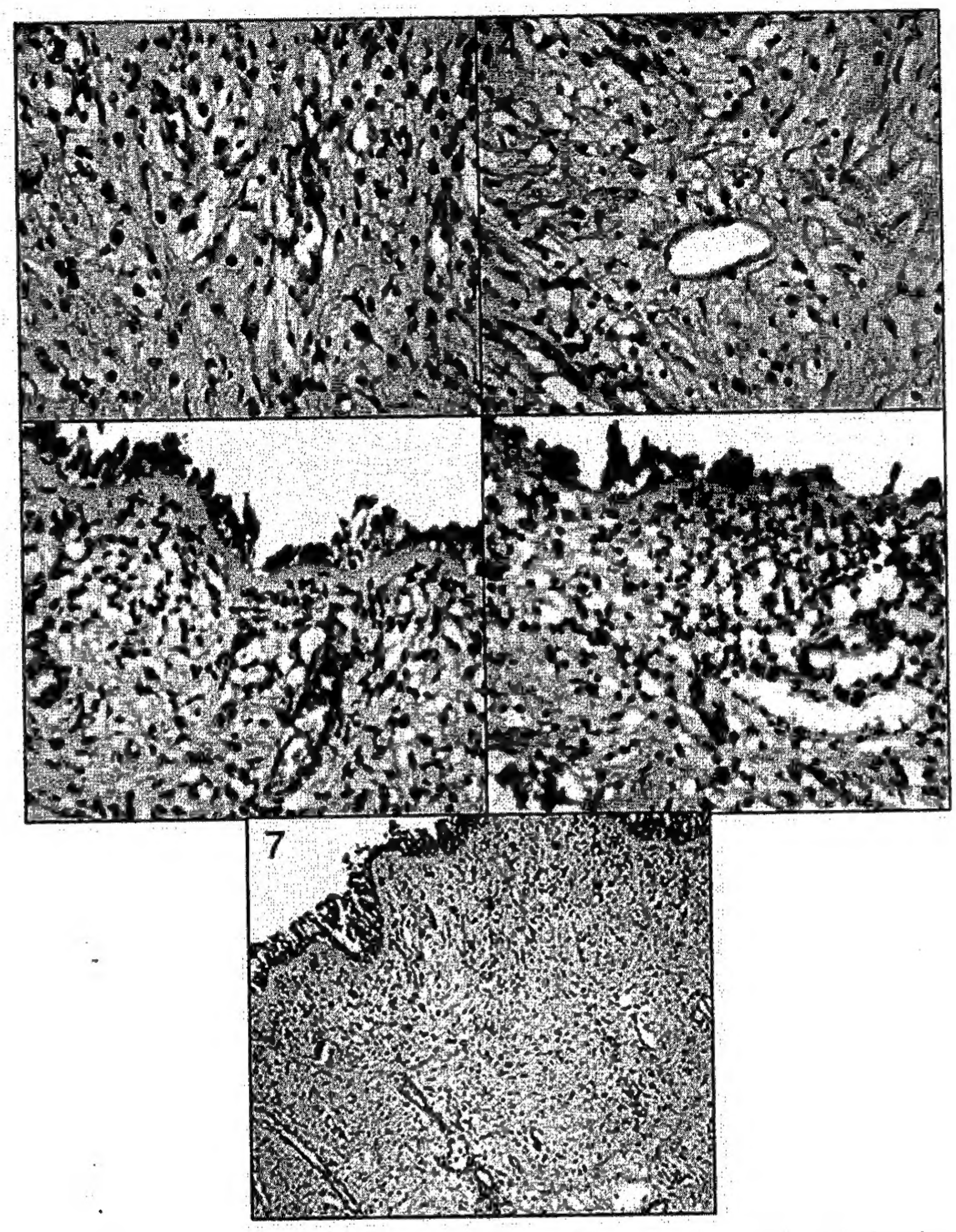

Fig 3 : A sinonasal polyp showing grade 3 CD95 - positive cells (browmish cytoplasmic staining) .

Fig 4 : A sinonasal polyp showing grade 1 CD95 - positive cells (browmish cytoplasmic staining) .

Fig 5 : A sinonasal polyp showing grade 3 CD68 - positive macrophages (browmish nuclear staining) .

Fig 6 : A case of sinonasal polyp showing grade 1 CD68 - positive macrophages . (Immunoperoxidase [IP] x 400) .

Fig 7 : A case of sinonasal polyp showing CD95 - positive inflammatory cells more frequently towards – the – periphery than in the central part of the lesion (IP x 100) .

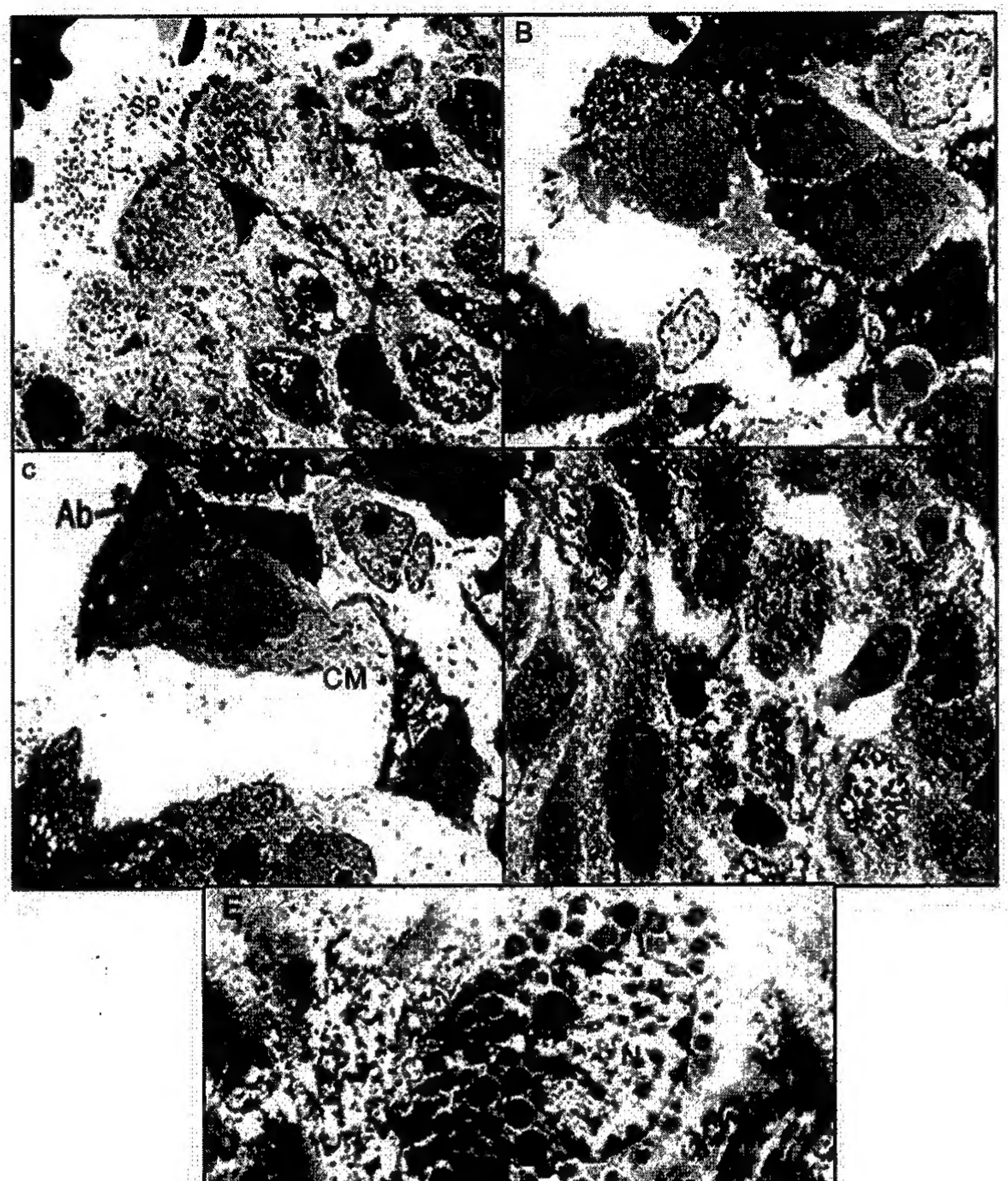

Fig . 8 : Electron micrographs showing cytolytic eosinophils detected by incomplete chromatolysis of the nucleus , disrupted cell membrane and spilling of protein – containing granules (SP) ; 8A . Apotოtic eosinophils revealed condensed chromatin (apoptotic body ; Ab ; 8A-D) and disrupted cell membranes (CM ; blue arrow head ; 8B & C) . Secondarily necrotic eosinophils displayed a condensed dark nucleus and damaged cell membrane (CM ; 8A-C) . The apoptotic eosinophils revealed electron – dense granules (G ; 8E) .

(Double fixation & Double stain : 8A-D x 10,000 and 8E x 12,000) .

Discussion

Sinonasal polyp is an upper airway inflammatory disease in which the structural modifications of epithelium (secretory hyperplasia and squamous metaplasia) and lamina propria (basement membrane thickening , extracellular matrix accumulation and collagen deposition) are associated with inflammatory cell infiltration (Molet *et al* ., 2003) .

The uniform handling of the tissue and the immediate fixation minimized the risk that the number of cells which might enter apoptosis after polypectomy would differ between cases . Compared to electrophoresis that demonstrates the apoptotic process after its point of no return (180 – bp fragmentation) , terminal - d - UTP nick end labeling assay (TUNEL) is less specific in this respect. The incorporation of nucleotides made by the TUNEL technique indicates DNA breaks of any size with free 3 – OH ends . Such breaks may occur early in the apoptotic process and at a stage when DNA repair is still possible . Therefore , TUNEL does not necessarily indicate that the individual cell will definitively die (Davidsson *et al.*,2000)

Our study revealed that the inflammatory infiltrate in the sinonasal polyps consisted of numerous eosinophils as well as a considerable number of lymphocytes and macrophages . Moreover , we displayed a variable grades of CD 95 expression on tissue eosinophils (regarding their average number) in 92 % of the cases as well as on numerous other inflammatory cells . These positive cells were seen more frequently towards the periphery compared to the central part of the polyps .

In addition , CD68 - positive cells (most probably macrophages) approximately equalled the number of eosinophils although their incidence varied from one polyp to another . All polyps contained CD 68 - positive cells in various grades regarding their average number .

Both CD 95 – and CD 68 – positivie cells were numerous in 58% & 56 % of cases respectively. Our findings were in agreement with those observed by Davidasson *et al* . (2000) and Henson et al . (2001) who stated that the Annexin – V positivity of eosinophils indicates that these cells signal their entry into apoptosis and the TUNEL positivity of eosinophils indicates that these cells have DNA breaks , which occur during the substantial period of the apoptotic process , and thus point to the apoptotic morphology in vivo . In addition , they reported that Annexin –V positivity demonstrates the switch of the membrane – bound phosphatidylserine molecule that occurs early in the apoptotic precess . They also demonstrated that many eosinophils are actively phagocytosed within the tissue and these cells signal, as well ,macrophage –like cells by the switch of phosphatidylserine molecule (binding of Annexin–V) which seems to be associated with the active phagocytosis by CD 68–positive cells .

Davidsson *et al* . (2000) stated that the further out toward the periphery of the polyp, the more pronounced the apoptosis (TUNEL positivity) and also the phagocytosis .

Steroids are known to reduce the survival rate of human eosinophils. Eosinophil apoptosis is likely an indirect effect of the elimination of survival – inducing cytokines caused by steroids. Also Fas ligand / Fas receptor interactions are suggested to be involved in the regulation of apoptosis and defects in this system could contribute to the accumulation of these cells in diseases (Matsukura et al ., 1997) . This statement may interpret the absence of eosinophil CD 95 immunoreactivity in 14 % of our cases .

Hebestreit *et al.* (1996) has been shown that activation of Fas with specific anti–Fas antibodies resolves chronic eosinophilic tissue inflammation , whereby apoptotic eosinophils were identified within the nasal polyps by in – situ detection of DNA strand breaks . As all our patients had received a local steroid treatment , some of the apoptotic eosinophils were probably died due to this therapy . This is supported by Nonaka *et al* ., (1999); Linden *et al* . (2000) ; Belvisi (2004) and Ochkur *et al* . (2007) obsrvation that the airway steroids through inhibition of growth factors including IL – 5 , both in vitro and in vivo ,

should increase the incidence of eosinophil apoptosis. Lombardi and Passalacqua (2003) increasingly recognized that the harmful effects of tissue eosinophilia are either due to the inappropriate neutralization of their products or failure to quickly eliminate these cells from the tissues. Wooley *et al* . (1996) & Duncan *et al* . (2003) postulated that eosinophil apoptosis may occur in vivo , thus , promoting the removal of airway eosinophils and the resolution of inflammation in asthma .

Ultrastructurally, we demonstrated a distribution of eosinophils in the epithelium and subepithelial layer of sinonasal polyp . Our finding revealed that the most common mechanism for eosinophilic elimination was apoptosis that was encountered in 73% of TEM – examined eosinophils about third of which displayed features of secondary necrosis whileas cytolysis as another mechanism was found in only 4 % of our TEM – examined cells . In addition , the macrophages were seen engulfing eosinophils . These ultrastructural findings confirmed the immunohistochemical ones and were in coinciding with those observed by Nutku *et al* . (2003) ; Gardai *et al* . (2003) ; Uller *et al.* (2004) and Erjefalt *et al.* (2004) . In contrast , Erjefalt and Persson (2000) and Farahi *et al* . (2007) found that the apoptotic eosinophils are exceedingly rare in human nasal polypous tissues in vivo and suggested the possibility that turnover of human airway tissue eosinophils in vivo , irrespective of steroid therapy , largely involves other mechanisms than apoptosis . Alternative modes include non – injurious egression between epitheial cells into the airway luman and proinflammatory disintegration through cytolysis .

Uller *et al* (2001) mentioned that cytolysis of eosinophils is a significant event that should not be confused with eosinophilic necrosis which occurs in vitro secondary to apoptosis and may also occur in vivo in airway tissues under the exceptional conditions caused by anti – Fas treatment of mouse airway .

Saunders *et al.* (1999) and Davidsson *et al.*(2000) reported that TUNEL technique or phosphatidylserine (Annexin) staining of the histologic sections would predictably produce false– positivie results because any section–induced cell damage would make the intracellular phosphatidylserine amenable for staining . Also , depending on the concentration and incubation time of involved enzymes and nucleotides , TUNEL method may fail to stain even truely apoptotic cells or , more commonly , may stain virtually all cells in the tissue irrespective of apoptosis .

Phagocytes taking up apoptotic cells may employ a range of possible recognition mechanisms (Davidsson *et al* . 2000) . The apoptosis and phagocytosis of eosinophils by macrophages , as detected in our study , have also described in vivo in human allergen – induced cutaneous late – phase reactions (Ohta and Yamashita ,1999). In – vitro studies have displayed that apoptosis of eosinophils is activated in a sequential manner and that eosinophils are phagocytosed as intact cells by macrophages (Simon and Alam , 1999) .

Davidsson *et al* . (2000) mentioned that eosinophils are phagcytosed by macrophage– like cells (CD 68- positive cells) within the polypoid tissue presumably after the phosphatidylserine switch attracts the macrophages. Thrombospondin is another recognition pathway for phagocy-tosis although it had not been detected in any of their studied polyps; Thus, they believed that the phosphatidylserine switch was to occur early in the apoptotic process and probably before the thrombospondin bindings .

Human eosinophils , 100 – fold more common in tissue than in blood , may therefore be the target for cytotoxic T – lymphocytes in tissue (Vignola *et al* ., 1999) . The Fas receptor (CD 95 ; death receptor) is likely a universal receptor , present on many human cell types and upregulated by certain stimuli when needed (Sorkness *et al* ., 2007) .

Presence of numerous inflammatory cells expressing CD95 within the polypous tissue in our as well as in Davidsson *et al* . (2000) and Henson *et al.* (2001) studies may well imply that the high number of apoptotic cells is due partly to Fas– induced apoptosis .

Conclusion

This study added further information regarding tissue eosinophilia , apoptosis and engulfment of eosinophils by macrophages in sinonasal polyps , Moreover , it concluded that the removal of eosinophils

by cytolysis , apoptosis and phagocytosis in the sinonasal polyps might be important mechanisms , particularly the later two , for the clearance of these cells from the respiratory tissue and therefore could improve the allergic and hypersensitivity manifestations .

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دور التساقط الخلوي والتهام خلايا الإيوسين في السليلة المخاطية للجيوب الأنفية : دراسة هستوكيميائية مناعية وتركيبية عالية الدقة

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تختفي خلايا الالتهاب من أنسجة المجري الهوائي عن طريق التساقط الخلوي والالتهام بالخلايا الآكلة ويمكن التخلص أيضاً من هذه الخلايا عن طريق التحلل الخلوي الأولي والموت الثانوي للتساقط الخلوي أو هجرة الخلايا عبر النسيج الظهاري . وقد قيمت هذه الدراسة دور التساقط الخلوي والتهام خلايا الأيوسين في السليلة المخاطية للجيوب الأنفية والتي استئصلت من خمسين مريضاً . وبالطريقة المناعية الهستوكيميائية تم تحديد كمية الخلايا الإيجابية لسي دي 95 وسي دي 68 عن طريق حساب متوسط العدد في عشر مجالات عشوائية عالية التكبير وكان النسيج الالتهابي المتسرب متضمناً العديد من خلايا الأيوسين وعدد لا بأس به من الخلايا الليمفاوية والعديد من الخلايا الآكلة . وقد تم أيضاً إظهار سي دي 95 بصورة متكررة علي خلايا الأيوسين وكذلك إظهاره علي العديد من الخلايا الالتهابية الأخرى أما الخلايا الإيجابية لسي دي 68 (وهي غالباً الخلايا الأكالة) فقد تساوت تقريباً في عددها مع خلايا الأيوسين ووجد بالطريقة التركيبية عالية الدقة أن 73 ٪ من عدد خلايا الأيوسين المفحوصة تعاني من تساقط خلوي (365 من 500 خلية) كان ثلثها تقريباً (89 من 365 بنسبة 24.3 ٪) يعاني من الموت الثانوي للتساقط الخلوي وفي المقابل كان 4 ٪ فقط من خلايا الأيوسين (بعدد 20 من 500 خلية) متحللة ذاتيا وقد تأكد بهذه الطريقة حدوث التهام خلايا الأيوسين بالخلايا الأكالة . وعلي ذلك نستنتج من هذه الدراسة أن التخلص من خلايا الأيوسين يتم غالباً عن طريق التساقط الخلوي والتهام الخلايا وهذا قد يكون مهماً في تطهير النسيج التنفسي من خلايا الأيوسين وبذلك قد تتحسن مظاهر الحساسية .